PATENT
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Applicant:	DONG, Zheng Xin	Art Unit:	1653
Serial No.:	09/857,636	Examiner:	LUKTON, David.
Filed:	November 2, 2001		
Title:	ANALOGUES OF GLP-1		

Commissioner of Patents
Washington, D.C. 20231

Sir:

DECLARATION UNDER 37 C.F.R. § 1.132

I, John E. Taylor, declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that willful false statements may jeopardize the validity of this application or any patent issuing thereon; and I further declare that:

1. I am the Associate Director of Receptor & Cell Biology at Biomeasure, Incorporated, of Milford, Massachusetts. I was awarded a Bachelor of Science degree in Zoology from Brigham Young University of Provo, Utah, in 1971, a Master of Science degree in Pharmacology in 1974 and a Doctorate of Philosophy degree in 1977 from the University of the Pacific of Stockton, California. I have been employed by Biomeasure, Incorporated, from 1983 to

the present. Part of my responsibilities as Associate Director of Receptor & Cell Biology is to supervise the performance of receptor binding assays of compounds at Biomeasure, Incorporated.

2. I am familiar with the Office Action mailed June 8, 2004, wherein the Examiner has rejected claims 10, 11, 12, 13, and 14 of the above-identified application.

3. Under my supervision, [$Aib^{8,35}$]hGLP-1(7-36)NH_2 (Applicant's compound of claim 10, hereinafter "Test Peptide") and native hGLP-1(7-36)NH_2 (hereinafter "Reference Peptide") were tested in the receptor binding assay described in paragraphs 4. and 5., below.

4. Cell Culture. RIN 5F rat insulinoma cells (ATCC-# CRL-2058, American Type Culture Collection, Manassas, VA), expressing the GLP-1 receptor, were cultured in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal calf serum, and maintained at about 37 °C in a humidified atmosphere of 5%CO_2 / 95% air.

5. Radioligand Binding. Cell membranes were prepared for radioligand binding studies by homogenization of the cells in 20 ml of ice-cold 50 mM Tris-HCl with a Brinkman Polytron (Westbury, NY) (setting 6, 15 sec). The homogenates were washed twice by centrifugation (39,000g/10 min), and the final pellets were resuspended in 50 mM Tris-HCl, containing 2.5 mM $MgCl_2$, 0.1 mg/ml bacitracin (Sigma Chemical, St. Louis, MO), and 0.1% bovine serum albumin ("BSA"). For assay, aliquots (0.4 ml) were incubated with 0.05 nM (^{125}I)hGLP-1(7-36)NH_2 (~2200 Ci/mmol, New England Nuclear, Boston, MA), with and without 0.05 ml of unlabeled competing Test Peptide or Reference Peptide. After a 100 min incubation (25 °C), the bound (^{125}I)hGLP-1(7-36)NH_2 was separated from the free by rapid filtration through GF/C filters (Brandel, Gaithersburg, MD), which had been previously soaked in 0.5% polyethyleneimine. The filters were then washed three times with 5 ml aliquots of ice-cold 50 mM Tris-HCl, and the bound radioactivity trapped on the filters was counted by gamma spectrometry (Wallac LKB, Gaithersburg, MD). Specific binding was defined as the total (^{125}I)hGLP-1(7-36)NH_2 bound minus that bound in the presence of 1000 nM hGLP-1(7-36)NH_2 (Bachem, Torrence, CA). Binding data were analyzed by computer-assisted nonlinear regression analysis (Data Analysis Toolbox, v.1.0, Molecular Design Limited, San Leandro, CA) and Inhibition Constant (Ki) values were calculated using the equation of Cheng and Prusoff (Cheng Y., Prusoff W. H., Biochem. Pharmacol. 22: 3099-3108, 1973).

6. Results: The Binding Constants for the Test Peptide and the Reference Peptide, as determined by foregoing assay, are:

	Ki (nM)
hGLP-1(7-36)NH_2	0.145 nM
[Aib^{8,35}]hGLP-1(7-36)NH_2	0.205 nM

7. Conclusion: The results of the Radioligand Binding assay described hereinabove demonstrate that [Aib^{8,35}]hGLP-1(7-36)NH_2 binds to the GLP-1 receptor with substantially the same affinity as hGLP-1(7-36)NH_2.

Date: 9/7/04

John E. Taylor